Anti-ATF6 α antibody, mouse monoclonal (1-7)

73-500, 50 μg

ATF6 (**a**ctivating **t**ranscription **f**actor **6**) is an endoplasmic reticulum (ER) membrane-bound transcription factor activated in response to ER stress. When unfolded proteins accumulate in the ER, **ATF6** is cleaved by regulated intramembrane proteolysis. The resulting amino-terminal fragment translocates to the nucleus and activates transcription by binding to ER stress-response elements present in the promoter regions of ER stress-inducible genes including those encoding ER chaperones and components of ER-associated degradation. **ATF6** consists of two closely related factors, **ATF6α** and ATF6β, in mammals. **ATF6α** but not ATF6β plays a pivotal role in transcriptional control.

The monoclonal antibody was characterized in the laboratory of Professor Kazutoshi Mori of Kyoto University. The antibody was produced from hybridoma cultured in serum-free medium and purified under mild conditions by propriety chromatography processes.

Applications: (Detailed Protocol is given below)

1. Western blotting
2. Immunoprecipitation (IP)

This antibody does not work for immunofluorescence analyses.

Immunogen: Recombinant ATF6α (His-tagged amino-terminal fragment of ATF6α)
Epitope: not determined
Isotype: mouse IgG2a κ
Form: purified monoclonal antibody (IgG) 1mg/ml in PBS, 50% glycerol, filter-sterilized
Specificity: specific to human ATF6α, no cross reactivity with mouse ATF6α
Storage: -20℃ (long period, -70℃)

Data Link

Swiss-Prot P18850

References

1. Hai T *et al* (1989) "Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers." *Genes Dev* **3**: 2083-2090 PMID 2516827
2. Haze K *et al* (1999) "Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress". *Mol Biol Cell* **10**: 3787-3799 PMID: 10564271
3. Yamamoto K *et al* (2007) "Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1". *Dev. Cell* **13**: 365-376 PMID: 17765680

SCETI K.K.
3-6-7 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013, JAPAN
Tel +81(3) 5510-2347 Fax +81(3) 5510-0133
URL: http://www.sceti.jp/export/ e-mail: exp-pet@sceti.co.jp

Protocol for ATF6α analysis using anti-human ATF6α monoclonal antibody

Both endogenous precursor ATF6α, pATF6α(P), and its cleaved product, pATF6α(N), can be detected in human cells such as HEK293T, HEK293 and HeLa cells by western blot analysis using anti-human ATF6α monoclonal antibody clone 1-7 (Fig. 1), according to the procedures described below. Clarity of the results may depend on cell types and culture conditions. If clear results could not be obtained by western blot analysis as seen with HeLa and HEK293 cells (Fig.1 right panel), it is worth while trying immunoprecipitation followed by western blot analysis according to the procedures described below.

Fig.1 Western blot analysis of human cell extracts using this antibody: Conversion of pATF6α(P) to pATF6α(N) in DTT- or tunicamycin-treated cells.

Tm: 2 μg/ml tunicamycin (inhibitor of N-glycosylation). DTT: 1mM dithiothreitol (reducing reagent).
The asterisk denotes an unglycosylated form of pATF6α (P).
ATF6α is constitutively expressed as pATF6α(P) (~90-kDa protein), and converted to pATF6α(N) (>50-kDa protein) in ER-stressed cells.

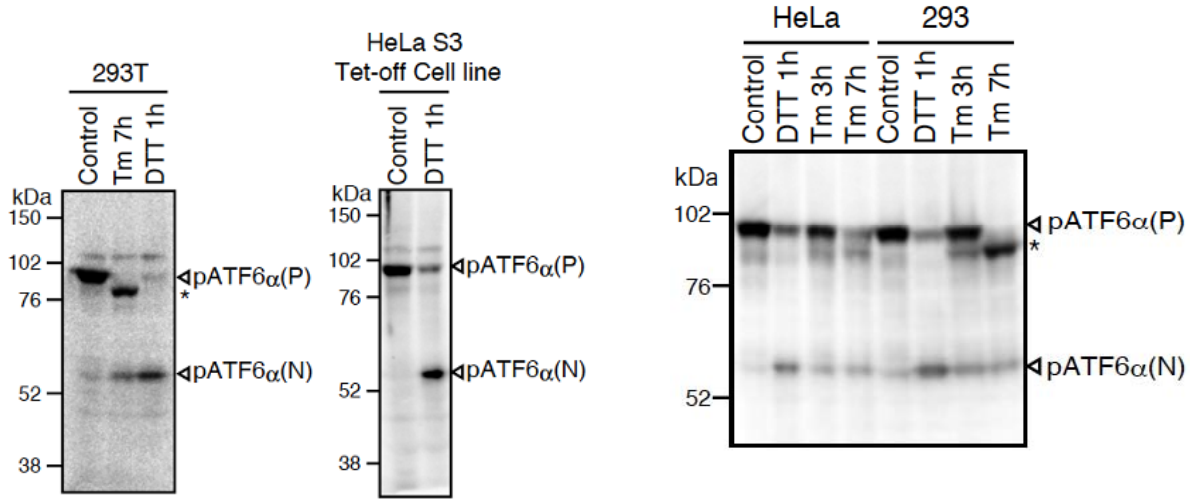
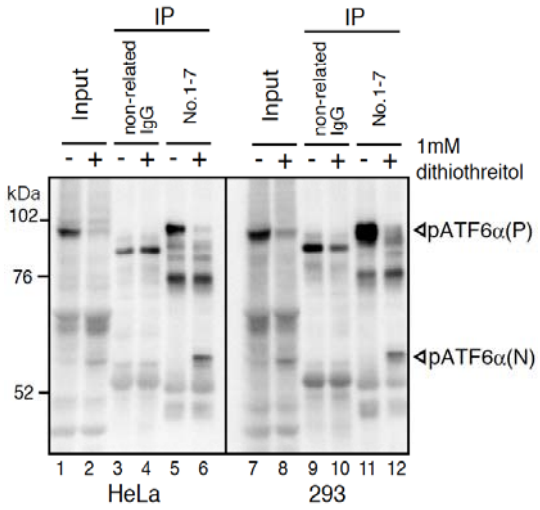


Fig.2 IP-Western blot analysis of human cell extracts using this antibody.

ATF6α was detected by Western blot (Input; lanes 1, 2, 7, and 8) using this antibody (No.1-7). After immunoprecipitation (IP) with non-related IgG (IP; lanes 3, 4, 9, and 10) or this antibody (No. 1-7) (IP; lanes 5, 6, 11, and 12), samples were subjected to SDS-PAGE and analyzed by Western blot using this antibody (# 1-7) and anti-mouse IgG antibody (light chain specific).

Detection of pATF6α(P) and pATF6α(N) is better in IP-Western blotting than Western blotting.



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Western blotting

SDS-sample buffer: 50 mM Tris/HCl, pH6.8, containing 2% SDS, (100 mM DTT), 10% glycerol and BPB

PBST: PBS containing 0.1% Tween 20

Blocking buffer: PBS containing 0.1% Tween 20 and 5% skim milk

・Sample Preparation (for HeLa or HEK293 cells cultured in 6cm dish)

(1) Wash cells with ice-cold PBS.

(2) Scrape cells in 500 µl of ice-cold PBS (+ protease inhibitor cocktail and 10 µM MG132) 2 times and collect cells by centrifugation at 5,000 rpm for 2 min.

(3) Lyse cells directly in 100 µl of SDS-sample buffer without reducing reagent (+ protease inhibitor cocktail and 10 µM MG132).

(4) Voltex mix vigorously.

(5) Boil the lysate for 5 min and voltex well.

(6) If the lysate is still viscous, boil again and voltex mix vigorously.

(7) Centrifuge at 14,000 rpm for 2 min.

(8) Determine protein concentration using BCA protein assay kit.

・SDS-PAGE and incubation with antibody

(9) Add one-tenth volume of 1 M DTT and boil for 5 min.

(10) Subject 50 µg of the lysate to 8% SDS-PAGE.

(11) Transfer to nitrocellulose membrane (such as Hybond-ECL, GE Healthcare).

(12) Incubate the membrane in Blocking buffer overnight at 4˚C.

(13) Incubate the membrane with primary antibody diluted in Blocking buffer (1:500-1:1000) for 1 h at room temperature or overnight at 4˚C. Wash the membrane 3 times each for 5 min with PBST.

(14) Incubate the membrane with HRP-conjugated secondary antibody for 1 h at room temperature. We recommend “ECL anti-mouse IgG, Horseradish Peroxidase linked F(ab’)2 fragment” (GE Healthcare NA9310V-1ML) or “Peroxidase-conjugated AffniPure Goat Anti-Mouse IgG, Light Chain Specific” (Jackson ImmunoReseach 115-035-174).

(15) Wash the membrane 3 times each for 5 min with PBST.

(16) Detect signals using an appropriate luminescent reagent.

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Immunoprecipitation

(For HeLa or HEK293 cells cultured in 6cm dish)

Lysis buffer: 50 mM Tris-HCl pH7.5, containing 150 mM NaCl, 1% NP-40, protease inhibitor cocktail and 10 μM MG132)

(1) Wash cells with ice-cold PBS, suspend them in 400 μl of Lysis buffer and stand on ice for 10 min.

(2) Clear the lysate by centrifugation at 14,000rpm for 10 min at 4˚C and transfer 300 μl of the supernatant to a new tube.

(3) Add anti-ATF6α antibody (1-3 μg) into the supernatant and incubate with gentle rotation for 2 h ~overnight at 4˚C.

(4) Add 30 μl of the 50% slurry of ProteinG-Sepharose suspended in Lysis buffer into the tube and incubate with gentle rotation for 1 h at 4˚C.

(5) Wash the Sepharose beads 2 times with Lysis buffer.

(6) Wash the Sepharose beads with PBS.

(7) Resuspend the Sepharose beads in 30 μl of SDS-sample buffer containing 100 mM DTT. Use 10 μl aliquot of the sample for Western blot analysis.

■ **Critical point**■

In IP-western blot analysis, use a light chain specific anti-mouse IgG antibody as a secondary antibody. “Peroxidase-conjugated AffniPure Goat Anti-Mouse IgG, Light Chain Specific” (Jackson ImmunoReseach 115-035-174) is recommended.

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